

# UK Patent Application GB 2 192 001 A

(43) Application published 31 Dec 1987

(21) Application No 8715050

(22) Date of filing 26 Jun 1987

(30) Priority data

(31) 79264

(32) 27 Jun 1986

(33) IL

(51) INT CL<sup>3</sup>  
C07D 209/32 A61K 31/40

(52) Domestic classification (Edition I)  
C2C 1343 213 220 22Y 250 251 25Y 300 30Y 315 31Y 324  
332 364 36Y 620 621 624 631 660 661 672 679 802 80Y  
AA LW  
U1S 2415 2417 C2C

(71) Applicant  
Ramat University Authority for Applied Research and  
Industrial Development Ltd,

(Incorporated in Israel),

32 University Street, Ramat Aviv, Israel

(72) Inventors  
Nava Zisapel,  
Moshe Laudon

(74) Agent and/or Address for Service  
Frank B. Dehn & Co, Imperial House, 15-19 Kingsway,  
London WC2B 6UZ

(56) Documents cited  
None

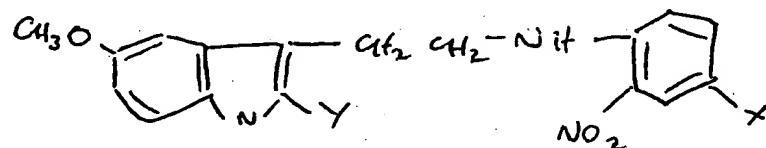
(58) Field of search  
C2C

## (54) Melatonin antagonist

(57) The invention relates to novel derivatives of melatonin, to the production of such new derivatives, to labelled forms, and especially radioactively tagged forms of such compounds, to assays for melatonin receptor function based on such radioactively tagged compounds and to pharmaceutical compositions which contain such novel non-labelled compounds.

The novel compounds are potent antagonists of melatonin which are of value in human medicine and also in animal husbandry.

The compounds are of formula:



where X is NO<sub>2</sub> or N<sub>3</sub>;  
and Y is H or I.

GB 2 192 001 A

## SPECIFICATION

## Melatonin antagonist

## 5 Field of the invention:

The invention relates to certain novel derivatives of melatonin (5-methoxy-N-acetyl-tryptamine), to the production of these, to labelled forms of these, and to pharmaceutical compositions containing as active ingredient such a derivative.

The novel derivatives are potent antagonists of melatonin and can be used for control of reproduction in vertebrate and for medical purposes. The radioactive labelled derivatives are valuable probes for use in biochemical and medical research and can be used as probes for the study of melatonin receptors.

## Background of the invention:

The biochemical role of melatonin has been described already in Chem Eng News, May 1, 1967, p. 40.

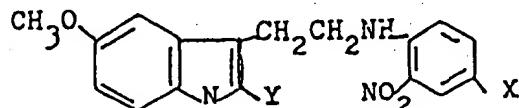
15 Melatonin is one of the principal hormones secreted by the pineal gland in all vertebrates. This gland expresses an overt rhythm of melatonin production which results in the nocturnal synthesis and secretion of melatonin into the blood. The melatonin rhythm provides a signal reflecting the changing environmental lighting cycle in the organism. A large body of evidence indicates that melatonin is involved in the coordination of timed physiological processes. This is evident in seasonal breeders such as hamsters and 20 sheep; the photoperiodic regulation of reproduction is manifested as a potent influence of melatonin on gonadal activity. Tornarkin et al, Science 227,714 (1985). It has been shown (Zisapel et al, Res. Comm. 104 (1982) and Brain Research 246 161 (1983) that melatonin inhibits the stimulated release of dopamine from rat hypothalamus. This provides means for the *in vitro* testing of putative melatonin antagonists.

## 25 Summary of the invention:

There are provided novel antagonists to melatonin. There are provided radioactively labelled probes for research into the activity of and as affinity label for the identification and isolation of melatonin receptors. There are further provided pharmaceutical compositions containing such melatonin antagonists as active ingredient. Such compositions are of use, for example, in animal husbandry in the breeding of fish, reptiles, 30 cattle and birds where they imitate in the organism the state of long photoperiods acting upon the reproductive system. They are of value in human medicine for the treatment of certain mental disorders and of endocrine disturbances. They are of value in the treatment of jet-lag, of manic depressive states, cluster headaches, sterility, and hypertension of the blind, of disturbances occurring in shift-workers, etc and in related phenomena which result from dysfunctions of the time-keeping system (biological clock) of the 35 organism.

The radioactively labelled derivatives may be used in order to study melatonin receptors, and in isolating these; they can be used in the study of mechanistic implications of melatonin action in timed physiological processes. Other and further features of the invention will become apparent hereinafter.

The novel compounds are derivatives of 5-methoxy-tryptamine and are of the following formula:



40  
45 wherein X is  $-NO_2$ ,  $-N_3$ ,  
Y is  $-H$ , I,  
as well as radioactive labelled compounds of this type, the labelling being advantageously  $^3H$ -labelled and the  $^{125}I$ -labelled derivatives of the dinitrophenyl derivative. The reaction of the invention comprises reacting 50 fluorodinitrobenzene or other corresponding compounds with a primary amine, such as 5-methoxytryptamine or a functional derivative thereof.

The iodo-derivative is prepared by reacting the dinitro-derivative with KI in the presence of Iodo-Gen. The azido derivative is prepared by reacting 5-methoxytryptamine with 4-fluoro-2-nitro azido benzene. The compounds of the invention are effective for use in veterinary medicine and in husbandry, as well as in 55 human medicine. The compounds can be administered orally and as such can be added to food or drinking water when used in animal husbandry. Amongst various effects observed, there can be mentioned, by way of example:

- 1) Long-term administration of melatonin (via the drinking water or via intraperitoneal injection) to prepubertal male rats results in retardation of testicular accessory sex organ development. Oral administration of ML-23 in the presence of melatonin entirely abolished melatonin's suppressive effects on prostate and seminal vesicle growth and on serum testosterone levels.
- 2) Melatonin administration to adult female rats in the afternoon of proestrus blocks the phasic secretion of luteinizing hormone and ovulation. Administration of ML-23 to female rats via the drinking water prevented the effect of melatonin and normal ovulation occurred.
- 3) Female rats kept under constant illumination become acyclical and exhibit persistent estrus characteris-

60 65

tics. Daily melatonin injections reestablish ovarian cyclicity in these rats but not in rats treated orally with ML-23.

4) Long-term administration of ML-23 alone to prepubertal male rats, enhanced testosterone levels in the blood of the animals.

5 The toxicity of the novel compounds was evaluated and it was found that they were found to be essentially non-toxic to mammals. Furthermore, body, testicular and ovarian weight of rats treated with the dinitro-compound for 4 weeks at a dosage of 800 µg/day was unchanged. 5

The novel active compounds are able to cross the blood brain barrier. Both derivatives reach the brain in intact form, as indicated by TLC of the labelled compounds extracted from the brain of rats which had been 10 injected the <sup>3</sup>H or the <sup>125</sup>I labelled analogs of the drugs 30 to 60 minutes prior to sacrifice. 10

The synthesis is a simple one, and the starting materials are inexpensive. Yields are high and pure products are easily obtained.

The following examples illustrate the preparation of derivatives according to the present invention:

15 **Example 1: N-2,4-dinitrophenyl-5-methoxy-tryptamine (ML-23)**

1 Mole of 5-methoxytryptamine was dissolved in 10 liters of water and the pH was adjusted to pH 8.3 with 2.5 moles of sodium bicarbonate (NaHCO<sub>3</sub>). A 1.5 % solution of fluoro-2,4-dinitrobenzene in 20 liters ethanol was added and the mixture was stirred during 2 hours at room temperature. The desired product precipitates out, it is washed and dried. The product is obtained in 90% yield, and TLC (chloroform, silica-gel plates, 20 reveals one yellow spot (*r*<sub>f</sub>=0.6) which is well resolved from the starting materials under the same conditions. 20

**Example 2: Production of N-2,4-Dinitrophenyl-5-methoxy-2-iodo-tryptamine**

The product of the preceding example was iodinated by reaction with potassium iodide Iodo-Gen. The main reaction product, 2-iodo-ML-23 was separated from unsubstituted material and purified by TLC (chloroform 25 on silica gel plates) as in the procedure described by Vakkuri et al (1984) for the iodination of melatonin. (*r*<sub>f</sub> of MLI-23=0.5, 60% Yield). The radioactive analogs of ML-23 and MLI-23 were prepared similarly, using <sup>3</sup>H-fluorodinitrobenzene and carrier-free Na125I (both from Amersham). 25

Azido-ML-23 was prepared from 5-methoxytryptamine (0.15 mole) by reaction with 4-fluoro 2-nitro azido benzene (0.3 mole) in 1 liter of 0.23 M sodium carbonate. The mixture was stirred overnight in the dark under 30 nitrogen atmosphere at 370. The major product (*r*<sub>f</sub>=0.5) was separated from other products and from unreacted materials by TLC (chloroform-methanol 1:1 on silica-gel plates), iodinated with Na 125I in the presence of iodogen and separated by thin layer chromatography (*r*<sub>f</sub>=0.8) in chloroform on silica-gel plates. 30

**Example 3: Melatonin assay using 2-<sup>125</sup>I-iodo-ML-23 (<sup>125</sup>I-MLI-23)**

35 Tissue sections or isolated cells were suspended in 10 ml/g ice-cold 0.32 M sucrose, homogenized in a teflon-glass homogenizer. The homogenate was centrifuged (10000 g-min) the pellet was discarded and the supernatant centrifuged (68000 g-min). The crude membrane pellet was suspended in 2 vol of 50 mM Tris-HCl buffer, pH 7.4 containing 4 mM CaCl<sub>2</sub>. 2-<sup>125</sup>I-iodo-ML-23 was prepared by iodination of ML-23 with Na<sup>125</sup>I (Amersham) (20 Ci/mmol) in the presence of Iodo-Gen. The principal iodination product, 2-<sup>125</sup>I-iodo-ML-23 (<sup>125</sup>I-MLI-23) was separated from unsubstituted ML-23 as described in Example 2. Aliquots of the 40 membrane preparations (200 µg protein/20 µl) were incubated with 40 µl Tris buffer containing 20–100 nM <sup>125</sup>I-MLI-23 for 30 min. at 37°C on a shaking water bath, in the absence or presence of unlabelled melatonin (50 nM). Membranes were then collected by vacuum filtration using GF/C glass fiber filters and washed with 3×4 ml buffer at 4°C. The filters containing bound <sup>125</sup>I-ML-23 were assayed for radioactivity using a Packard 45 gamma counter. Specific binding was defined as that displaced by 50 nM of non-radioactive melatonin and ranged from 50 to 60% of the total binding at 50 nM <sup>125</sup>I-MLI-23. 45

The equilibrium binding data could be fitted by non-linear regression to a single site model according to the following equation: B=Bmax/(1+Kd/L) where B and Bmax are the specific binding at different <sup>125</sup>I-MLI-23 concentrations (L) and at saturation, respectively, and Kd is the binding dissociation constant.

50 The distribution of melatonin binding sites in six discrete brain regions of female rats at the estrous stage and the effects of ovariectomy and subsequent 17p-estradiol treatment on these binding sites were studied using such procedure. Specific binding of <sup>125</sup>I-MLI-23 was found in the hypothalamus, medulla-pons, hippocampus, cerebellum, striatum and parietal cortex of the female rats. Ovariectomy produced a large estradiol-reversible decrease in <sup>125</sup>I-MLI-23 binding in the medulla-pons and hypothalamus. In contrast,

55 <sup>125</sup>I-MLI-23 binding sites in the other brain regions, were generally unaffected by ovariectomy or estradiol. The estradiol regulated changes in melatonin binding in the hypothalamus and medulla pons may reflect the role of a specific estradiol-melatonin interaction in coordination of the neuroendocrine reproductive axis. 55

**CLAIMS**

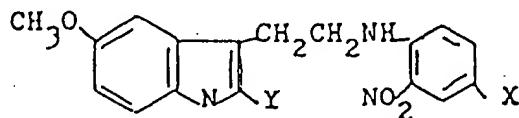
60

60

- Derivatives of 5-methoxy-tryptamine of the general formula:

5

5



wherein X is  $-NO_2$ ,  $-N_3$ ,

Y is  $-H$ ,  $I$ ,

and radioactively labelled forms of any of these.

- 10 2. A derivative according to claim 1, in the  $^3H$  or  $^{125}I$ -labelled form. 10
3. N-2,4-dinitrophenyl-5-methoxy tryptamine and its labelled forms.
4. N-2,4-dinitrophenyl-5-methoxy-2-iodo tryptamine and its labelled forms.
5. A pharmaceutical or veterinary composition containing as active ingredient an effective dosage of a compound defined in any of claims 1 to 4 (in the unlabelled form).
- 15 6. A pharmaceutical or veterinary composition according to claim 5, where the daily dosage is in the order of 0.2 mg/kg/day to about 2 mg/kg/day. 15
7. An assay for melatonin receptor function based on the use of a labelled form of a compound defined in claim 1.
8. Process for the production of the compounds defined in claim 1, substantially as hereinbefore described
- 20 and with reference to the Examples. 20

Printed for Her Majesty's Stationery Office by Croydon Printing Company (UK) Ltd, 11/87, D8991685.  
Published by The Patent Office, 25 Southampton Buildings, London WC2A 1AY, from which copies may be obtained.